Structural Investigation of Cholesterol-α-glucosyltransferase from *Helicobacter pylori*

Man-Ting Hsu (徐曼庭)[1],Tsan-Jan Chen (陳粲然)[1],and Wen-Ching Wang (王雯靜)[1*]

[1]Institute of Molecular & Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan

Abstract

Helicobacter pylori a microaerophilic Gram-negative bacterium, successfully colonizes onto the mucous layer of the human stomach. According to World Health Organization, approximately one-half of the worldwide human population is infected with this bacterium. *H. pylori,* inducing chronic inflammation in gastric mucosa and further progressing into gastric and duodenal ulcers, and even gastric cancer. Accumulated results indicate that cholesterol plays a very important role in the pathogenesis of *H. pylori*. Upon infection, *H. pylori* extracts cholesterol from host membrane and assimilates into glucoside derivatives including α-cholesteryl-glucoside (α-CG), cholesteryl-acyl-glucoside, and cholesteryl-phosphatidyl-glucoside. These derivatives are important for *H. pylori* to escape phagocytosis by macrophages, T cell activation, and bacterial growth. The cholesteryl glucosides are enriched in lipid rafts of host membrane to facilitate bacterial infection and internalization. The *H. pylori* enzyme, cholesterol-α-glucosyltransferase (αCGase), encoded by the *HP0421* gene is responsible for the synthesis of α-CG. Infection with αCGase-knockout *H. pylori* leads to a reduced degree in lipid-raft coalescence/restructuring and a decreased level of internal survival in macrophages. Yet, the structure-activity of the whole αCGase remains elusive. In this study, homology modeling reveals that αCGase consists of an N-terminal cholesterol binding domain and a C-terminal UDP-Glucose binding domain. Hydrophobicity plot shows that the predicted active-site surface of N-terminal cholesterol-binding domain is hydrophobic, which may facilitate its binding with cholesterol. We have expressed and purified the recombinant αCGase in *Escherichia coli* expression system. Size-exclusion chromatography analysis suggests that αCGase exists as oligomeric as wellas monomeric forms. Initial protein crystals were obtained from crystallization screening by using the monomeric αCGase form. Enzymatic activity assay shows that addition of detergent (0.1 % Triton X-100) can increase its catalytic activity. Future optimization of the protein crystals will be carried out to determine the crystal structure of αCGase as a foundation to develop novel anti-microbial strategies.

Keyword : Helicobacter pylori, cholesterol-α-glucosyltransferase, enzyme coupling assay, protein crystallography, homology modeling.